19 Virus-resistant Transgenic Papaya: Commercial Development and Regulatory and Environmental Issues

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Abstract

In Hawaii, transgenic papaya resistant to *Papaya ringspot virus* (PRSV) was developed starting in the 1980s and released commercially in 1998 to combat the widespread destruction of Hawaii's papaya industry. This review describes the proactive development of the transgenic papaya and its impact on stemming the destruction caused by PRSV in Puna, the main papaya producing area in Hawaii. It also focuses on the regulatory issues that were confronted in obtaining approval from the US government's Environmental Protection Agency (EPA), Animal Plant Health Inspection Service (APHIS) and Food and Drug Administration (FDA). The performance of the transgenic papaya is traced over the last 8 years following commercial release, with special observations on the issues of environmental impact and coexistence with non-transgenic papaya. The latter is quite important since a significant part of Hawaii's papaya is exported to Canada and Japan. Canada has approved the transgenic papaya, but efforts to get approval for export of transgenic papaya to Japan are still ongoing.

Introduction

Papaya (*Carica papaya* L.) is an important fruit crop in tropical and subtropical regions due to its economic, nutritional, industrial, pharmaceutical and medicinal values, for local and export markets. *Carica papaya*, a member of the Caricaceae family, probably originated from the Southern part of Mexico and the Northern region of Central America (Badillo, 1993). It is relatively easy to grow from seed. The first mature fruits can be harvested 9 months after sowing, and fruits are produced year-round. The papaya was disseminated into the Asian tropics during the 1600s by seeds taken to the Malay Peninsula, India and Philippines. Documents show a wide distribution in the Pacific Islands by the 1800s (Nakasone, 1975). The Food and Agricultural Organization (FAO) of the United Nations (UN)

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estimated that about 5.85 million tonnes of fruit were harvested in 2004, almost doubling the 1980 harvest. Brazil (21.6%), Mexico (13.1%), Nigeria (11.6%), Indonesia (11.1%) and India (10.1%) are the largest producers of papaya (FAO, 2004).

A major limiting factor for papaya cultivation worldwide is the disease caused by PRSV. Discovered in 1945, PRSV is the most widespread and damaging papaya virus. The name of the disease, papaya ringspot, is taken from the ringed spots on fruit of infected trees (Jensen, 1949). Trees infected with PRSV develop a range of symptoms: mosaic and chlorosis of leaf lamina, water-soaked oily streaks on the petiole and upper part of the trunk, distortion of young leaves that resembles mite damage, loss of vigour and stunting (Purcifull *et al.*, 1984). Plants infected at the seedling stage or within 2 months after planting do not normally produce mature fruit, while trees infected at later stages produce few fruits of poor quality, due to the presence of ringspots and generally lower sugar concentrations. PRSV is transmitted by numerous species of aphids in a non-persistent manner to a limited host range of cucurbits and papaya, and also produces local lesions on *Chenopodium quinoa* and *C. amaranticolor*. Evidence suggests that PRSV is not seed transmitted, although there has been a report of seed transmission. PRSV is grouped into two types: type P (PRSV-p) infects cucurbits and papaya; whereas type W (PRSV-w) formerly referred to as WMV-1 infects cucurbits but not papaya (Purcifull *et al.*, 1984).

Much progress has been made in the molecular characterization of PRSV. Strains of PRSV-p from Hawaii and Taiwan have been completely sequenced (Yeh *et al.*, 1992; Wang and Yeh, 1997). The genomic RNA consists of 10,326 nucleotides and has the typical array of genes found in potyviruses (Fig. 19.1A). The genome is monocistronic and is expressed via a large polyprotein that is subsequently cleaved to functional proteins. There are two possible cleavage sites, 20 amino acids apart, for the N-terminus of the coat protein (CP). Both of these sites may be functional; the upstream site for producing a functional NIb protein (the RNA-dependent RNA polymerase), and the other, to produce a CP that is capable of functioning in aphid transmission (Quemada *et al.*, 1990; Yeh *et al.*, 1992; Wang *et al.*, 1994). It is impossible to segregate PRSV-p and PRSV-w types by their CP sequences. Within the p types, however, the CP sequences can diverge by as much as 12%.

The Hawaii papaya industry started in the 1940s on the island of Oahu, on about 200 ha (Ferreira *et al.*, 1992). By the 1950s, production on Oahu was affected by PRSV and the industry moved to the island of Hawaii into the area of Puna, which had no PRSV nor commercial papaya production. Growing area increased to 263 ha by 1960 and to 911 ha in 1990. In contrast, the growing area on Oahu fell to less than 20 ha by 1990 (Ferreira *et al.*, 1992). The yellow-fleshed 'Kapoho' was the dominant papaya grown in Puna, distantly followed by the red-fleshed 'Sunrise'. In fact, Kapoho made up 95% of the state of Hawaii's papaya production in 1992.

Remarkably, despite the presence of PRSV in Hilo only 30 km away, Puna remained free of PRSV for over 30 years, thanks to a surrounding

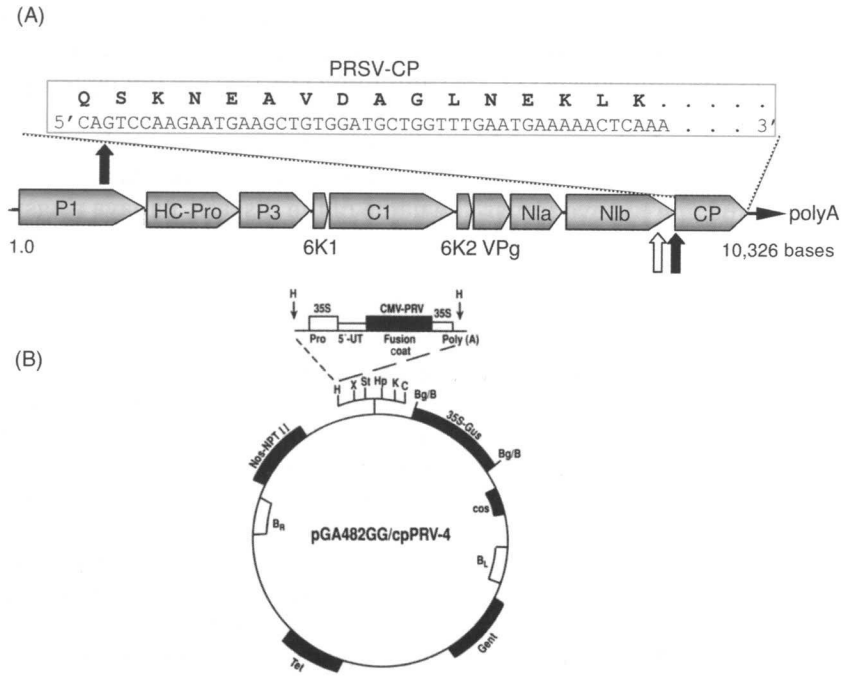

Fig. 19.1. (A) Organization and proteolytic protein products of the 10,326 base monocistonic PRSV genome. The N-terminal sequence of the PRSV HA 5-1 CP is shown at the top. Box arrows represent the proteolytic sites producing the mature CP. (From Tripathi *et al.*, 2006.) (B) Map of the functional genes of the *Agrobacterium* transformation vector pGA482GG/cpPRV-4 used for generating PRSV-resistant papaya. The PRSV *CP* gene cassette consists of the *CP* structural gene of PRSV HA 5-1 translationally fused to the N-terminal end of the cucumber mosaic virus CP (CMV-PRV) including the translation initiation codon, the CMV 5' untranslated sequence (5'UTR) and the Cauliflower Mosaic Virus 35S promoter (35S). The CMV-PRV gene cassette is flanked by selectable and visible marker genes, *nptII* and *uidA* (GUS), respectively. B_R and B_L are the left and right borders of the transformation vector T-DNA sequence. (From Ling *et al.*, 1991.)

area of sparsely populated lava which served as a physical barrier and to diligence by the Hawaii Department of Agriculture (HDOA) in surveying and rogueing infected trees in those nearby communities. However, it was highly probable that PRSV would someday be found in Puna. In 1978, one of the authors (D. Gonsalves) started research towards developing control methods for PRSV. The development of virus-resistant transgenic papaya was initiated in the mid-1980s following unsuccessful attempts at controlling the disease by non-biotechnological means. In 1992, PRSV was indeed discovered in Puna and papaya production there went from 24,045 t in 1992 to 12,134 t in 1998, the year transgenic papaya was commercialized and seeds were first released.

In this chapter, we will cover the development of the transgenic papaya, the environmental risks and regulatory issues involved in its commercialization and acceptance, and its impact on disease management. The readers are also referred to previous publications on the transgenic papaya case (Gonsalves, 1998, 2002, 2006; Gonsalves and Ferreira, 2003; Fermín *et al.*, 2004; Gonsalves and Fermín, 2004; Gonsalves *et al.*, 2006; Tripathi *et al.*, 2006).

Development of Transgenic Papaya

In the mid-1980s, an exciting, yet unproven alternative approach to control viral diseases was introduced. Transgenic tobacco expressing the *CP* gene of *Tobacco mosaic virus* (TMV) showed significant delay in the development of disease symptoms caused by TMV (Powell-Abel *et al.*, 1986). This approach, which provided protection against the detrimental effects of pathogens by expression of genes or sequences of the same or related pathogens, was coined 'Parasite-Derived Resistance' (now referred to as Pathogen-Derived Resistance or PDR) by Sanford and Johnston (Sanford and Johnston, 1985; Baulcombe, 1996; Baulcombe *et al.*, 1996; Beachy, 1997). Of interest, the resistance was genetically inherited, offering a potentially effective and feasible way for controlling PRSV in papaya.

The mild Hawaiian PRSV strain HA 5-1 was used as the source of the *CP* gene for the transgene construct since the goal was to create papaya resistant to Hawaiian strains of the virus (Quemada *et al.*, 1990). The transgene was designed to allow the translation of the *CP* gene, as at that time, it was thought that the CP protein was required for PDR. Since the PRSV CP is produced by post-translational protease cleavage (Fig. 19.1A), there are no native translation signals specific for the *CP* sequence. Therefore, a chimeric gene was designed utilizing the translation signals found in the leader sequence of the *Cucumber mosaic virus* (CMV) *CP* gene fused in frame to the structural sequence of the PRSV *CP* (Fig. 19.1B) (Ling *et al.*, 1991). A key point to transforming a papaya plant is the development of tissue culture conditions, particularly for regeneration. Efforts to develop a papaya regeneration system were unsuccessful until a technique to produce highly embryogenic tissue starting from immature zygotic embryos was developed (Fitch and Manshardt, 1990). The biolistic approach (Sanford *et al.*, 1992) was used to transform papaya with the PRSV *CP* gene construct followed by selection and regeneration of kanamycin-resistant clones (Fitch *et al.*, 1990, 1992). The target cultivars were the red-fleshed Sunrise, Sunset (a sib selection of Sunrise) and the yellow-fleshed Kapoho. A total of 10 papaya plants positive for GUS activity as well as for the *CP* gene by polymerase chain reaction (PCR) amplification were obtained – five Sunset and five Kapoho (Fitch *et al.*, 1990, 1992).

Initial resistance evaluations of R_0 tranformants in the greenhouse and field

To determine the functionality of the PDR-based transgene system in papaya in a timely manner, screening for resistance was initially performed on the original transformants (R_0). Sufficient material for screening was accomplished through micropropagation to produce R_0 clones for greenhouse inoculation tests with the severe Hawaiian PRSV isolate HA. One *CP* gene-positive line tested, a transformed Sunset line designated 55-1, showed excellent resistance to PRSV HA (Fitch *et al.*, 1992).

The research also moved ahead aggressively to determine whether the promising line 55-1 (R_0 material) would be resistant to PRSV and have suitable horticultural characteristics under field conditions (Lius *et al.*, 1997). The experimental samples included micropropagated 55-1 R_0 plants, non-transformed Sunset and a transgenic line lacking the *CP* gene planted in University of Hawaii fields located in Waimanalo, on the island of Oahu. Inoculations were performed either manually or by vector transmission using an isolate found on Oahu. All of the non-transgenic plants became severely infected within 5 months and were completely decimated by the end of the trial, whereas line 55-1 remained symptomless throughout the trial period which lasted from 1992 to 1994.

Comprehensive greenhouse resistance evaluations for progeny (R_1) of promising line

R_1 progeny of 55-1 were created by crossing a 55-1 R_0 plant with non-transgenic papaya. Crossing R_0 55-1 was necessary since it turned out to be a female plant and thus progeny could not be obtained directly from selfing. The 55-1 R_1 seedlings had a 50% segregation ratio for the neomycin phosphotransferase protein, suggesting that the transgene *nptII* insert and presumably the linked *CP* gene was present as a single copy in the parental R_0 line.

R_1 plants were used to screen for resistance to PRSV isolates from Hawaii and other regions under greenhouse conditions. Resistance of the 55-1 R_1 plants was tested against PRSV isolates from Mexico, Florida, Bahamas, Australia, Brazil, China, Okinawa, Ecuador, Guam, Thailand, Jamaica and Hawaii (Tennant *et al.*, 1994). Isolates from Guam, Brazil, Thailand, Ecuador and Okinawa induced severe symptoms on all transgenic plants, although the symptoms were not as severe as those observed on non-transgenic plants. Isolates from Australia, China and Jamaica induced an attenuated phenotype on all transgenic test plants. Excellent resistance was found for Hawaii isolates. A fraction of the plants infected with virus strains from the Bahamas, Mexico and Florida exhibited severe phenotypes whereas others were symptomless. Symptomless plants remained symptomless following reinoculation.

Development of cultivars 'SunUp and Rainbow'

The R_0 transgenic line 55-1 served as a source of germplasm to create SunUp and Rainbow, papaya destined to become commercial cultivars.

As mentioned previously, the 55-1 R_0 plant was a transgenic Sunset, which is a commercial red-fleshed cultivar. The SunUp variety, which is homozygous (*CP*/*CP*) for the transgene, but is otherwise identical to Sunset, was created as the R_3 generation of the original transformant 55-1. This germplasm held the hope for the development of new resistant varieties since 100% of the progeny from crosses with any other non-transgenic variety would be hemizygous for the *CP* gene (*CP*/+). In Hawaii, the yellow-fleshed Kapoho variety is by far the most popular among farmers and consumers and has a pyriform shape and medium size, which are desirable commercial characteristics for packing and shipping. Thus, in attempt to combine the PRSV resistance and Kapoho characteristics, Rainbow, an F_1 hybrid between SunUp and Kapoho, was created (Manshardt, 1999). The resulting Rainbow cultivar bore pear-shaped fruit with yellow-orange flesh as anticipated and was hemizygous for the transgene (*CP*/+).

Performance of Transgenic Cultivars

Field trial of SunUp and Rainbow in Puna

By 1994, the complete devastation caused by PRSV in Kapoho, a major papaya producing area of Puna, created a critical situation for survival of the Hawaii papaya industry. At the same time, the results from the R_0 field trial on Oahu were quite encouraging. A field trial was conducted in Kapoho to determine if the transgenic papaya could be used to rescue the papaya industry. By late 1994, an application for a field trial was submitted to APHIS. Approval was obtained in early 1995 and the field trial was set up in Kapoho in October 1995 (Ferreira *et al.*, 2002). The field trial was allowed with the stipulation that (i) the field must be sufficiently isolated from commercial orchards to minimize the chance of transgenic pollen escaping to non-transgenic material outside of the field test; (ii) all abandoned papaya trees in the area must be monitored for the introgression of the transgene into fruits of these trees; and (iii) all fruits had to be buried on site.

The results from the field trial (Fig. 19.2) clearly demonstrated the potential value of the transgenic papaya (Gonsalves, 1998; Ferreira *et al.*, 2002). Except for three plants that showed infection at the beginning of the trial, none of the transgenic plants became infected. In contrast, 50% of the non-transgenic control plants were infected within 5 months after transplanting while all were infected by 7 months. Rainbow averaged about 112,082 kg/ha/year of marketable fruit during the trial, a higher yield compared to the average production from non-infected Kapoho, whereas the non-transgenic plants averaged about 5604 kg/ha/year. In addition to evaluating Rainbow for PRSV resistance, it was also critical to analyse its fruit for taste, production, colour, size, and packing and shipping qualities as it was targeted as the alternative variety to Kapoho. The consensus was that Rainbow is a more than adequate substitute for Kapoho even though Rainbow has a slightly larger fruit size.

Fig. 19.2. Aerial view of the transgenic papaya field trial in Puna, Hawaii. At the centre is a block of Rainbow plants surrounded by non-transgenic Sunrise, which are stunted due to PRSV infection. Adjacent to the field at the upper right was a similar block consisting of a non-transgenic version of Rainbow (F_1, Sunset × Kapoho) and a transgenic line similar to Rainbow but with a distinct transgene insertion. The open area in the lower right foreground is the position of the abandoned, PRSV-infected papaya field used as the source of virus inoculum and cleared prior to flowering of the experimental field. (From Tripathi *et al.*, 2006.)

Greenhouse evaluation of Rainbow and SunUp: the effect of transgene copy number, plant development and coat protein homology

As noted above, earlier greenhouse work revealed that the resistance of R_1 plants of line 55-1 was narrow in that they were resistant to PRSV isolates from Hawaii but largely susceptible to isolates outside of Hawaii. In follow up greenhouse studies (Tennant *et al.*, 2001), Rainbow showed similar narrow resistance as R_1 plants of line 55-1 (Table 19.1). In contrast, SunUp showed resistance to Hawaii isolates and to isolates from Jamaica and Brazil. It thus appeared that *CP* gene dosage affected the broadness of resistance since SunUp is homozygous and Rainbow hemizygous for the *CP* gene. Further studies also showed that plant development (age and height) made a difference in that Rainbow at a young age showed variable resistance to Hawaii isolates but complete resistance as plants were older and larger. Likewise, the young SunUp were susceptible to the Thailand isolate but older ones were resistant or showed a long delay in symptom expression.

Table 19.1. *CP* nucleotide sequence homologies of PRSV isolates to PRSV HA 5-1 and summary of reactions of isolates inoculated to Rainbow and SunUp papaya. (From Tripathi *et al.*, 2006.) (Modified from Tennant *et al.*, 2001.)

	% Homology to transgene *CP*					Reaction to isolates	
PRSV isolates	N	core	C	3' ncr	overall	Rainbow	SunUp
Hawaii-HA	99.3	99.8	100	100	99.8	R	R
Hawaii-OA	97.3	98.0	100	95.7	97.9	sR	R
Hawaii-KA	95.3	97.1	98.3	93.6	96.7	sR	R
Hawaii-KE	95.3	97.1	98.3	93.6	96.7	sR	R
Jamaica-JA	89.3	95.0	91.5	69.6	92.5	S	R
Brazil-BR	84.4	93.9	98.3	73.3	91.6	S	R
Thailand-TH	83.7	90.7	91.5	89.4	89.5	S	sR

Rainbow and SunUp are hemizygous (*CP*/+) and homozygous (*CP*/*CP*), respectively, for the PRSV HA 5-1 *CP* transgene. N = 199 nucleotides of the amino terminus, core = 641 nucleotides of the core region, C = 59 nucleotides of the carboxy terminus and 3' ncr = 35 nucleotides of the non-coding regions following the stop codon. R = resistant. sR = susceptible at young stages and resistant at older stages. S = susceptible.

Comparison of the *CP* gene sequences from the various isolates suggested that resistance is affected by *CP* homology to the transgene, with the resistance being strongest against PRSV isolates with the highest homology to the transgene. The *CP* genes of Hawaiian PRSV isolates showed 97–100% homology to the transgene *CP*, while the *CP* genes of other isolates (Jamaica, Brazil, Thailand) showed 89–93% homology. The *CP* gene of the Thai PRSV isolate had the least homology to the transgene *CP*. The above observations are consistent with a resistance mechanism based on homology-dependent post-transcriptional gene silencing (PTGS). Lastly, nuclear run-on experiments confirmed PTGS (Tennant *et al.*, 2001).

Deregulation and Commercialization of SunUp and Rainbow Papaya

In this section, we discuss the steps leading to the deregulation of Rainbow and SunUp in the USA, detailing the roles of the various federal agencies in this process. The timetable of events is shown in Table 19.2. The USA has a coordinated, risked-based system for ensuring that new biotechnology products are safe for the environment based on a policy 'Coordinated Framework for Regulation of Biotechnology', established in 1986 (PEW, 2004). The policy is carried out by three federal agencies: APHIS, the regulatory arm of the US Department of Agriculture (USDA); the EPA; and the FDA. A new web site with coordinated information from the three federal agencies including a database of completed reviews of genetically engineered (GE) crops can be found at http://usbiotechreg.nbii.gov/. The role of the state in regulation of genetically modified organisms (GMO), in

Table 19.2. History of development and deregulation of SunUp and Rainbow in the USA. (Modified from Tripathi *et al.*, 2006.)

Year	Event	Reference
1990	PRSV-resistant papaya R_0 line 55-1 hemizygous for the *CP* transgene is created by biolistic transformation.	Fitch *et al.* (1992)
1991	APHIS issues permit for field trial of 55-1 in University of Hawaii's experimental farm in Waimanalo.	
1992	Greenhouse evaluation of a R_1 line hemizygous for the CP transgene of 55-1	Tennant *et al.* (1994)
1992	First field trial of 55-1 transgenic papaya was conducted in Waimanalo on Oahu island. During this time cultivars Rainbow and SunUp hemizygous and homozygous, respectively, for the CP transgene found in 55-1 were developed.	Lius *et al.* (1994, 1997); Manshardt (1999)
1994	Initial consultation with the FDA	
1995	APHIS issues a permit for a second field trial. Field trial of SunUp and Rainbow began in Puna on Hawaii island	Ferreira *et al.* (2002)
1996	Transgenic line 55-1 and its derivatives were deregulated by APHIS	Gonsalves (1998); Strating (1996)
1997	Submission of safety and nutritional assessment of 55-1 to FDA	
1997	Exemption from EPA was granted	Gonsalves (1998)
1997	FDA approval was granted for the transgenic lines	Gonsalves (1998)
1998	Bulk seed production of SunUp and Rainbow was completed	Wenslaff and Osgood (1999)
1998	License agreements were obtained from all parties allowing the commercial cultivation of transgenic papaya and its derivatives in Hawaii only. Seeds were released to farmers.	Gonsalves (1998)

general, parallels that of federal regulations although the specifics differ between state to state (Taylor *et al.*, 2004).

APHIS is responsible for most issues relating to environmental safety and regulates GE crops as 'regulated articles', organisms and products known or suspected to be plant pests, or plant pest risks. This regulation is currently administered through the Biotechnology Regulatory Service (BRS) of APHIS. APHIS regulates the import, handling, interstate movement, release into environment including confined experimental use and field trials of GE crops under the Plant Protection Act (PPA) of 2000. Evaluation is based on: potential environmental impact for plant pest risk; disease and pest susceptibilities; the expression of gene products, new enzymes or changes to plant metabolism; weediness and impact on sexually compatible plants; agricultural or cultivation practices; effects on non-target organisms; and the potential for gene transfer to other types of organisms.

In reference to evaluation of papaya line 55-1 from which SunUp and Rainbow were derived, APHIS was largely concerned with the potential risk of heteroencapsidation, recombination, transgene flow to wild relatives and weediness of virus-resistant papaya. These aspects will be discussed in detail later, in the section on Environmental Risk Issues. In November 1996, transgenic line 55-1 and its derivatives were deregulated by a decision document and environmental assessment (EA) document concluding a 'Finding of No Significant Impact' (FONSI) from APHIS (Strating, 1996).

EPA, through its Biopesticides and Pollution Prevention Division of the Office of Pesticide Programmes, regulates the sale, distribution and use of pesticides to protect both human health (food safety) and the environment from pesticides. Under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), regulated pesticides include 'pesticidal substances' produced by plants and microbes (products of biotechnology). Pesticides produced by GE plants are referred to as biopesticides or plant-incorporated protectants (PIP) and are regulated by the EPA. However, biopesticides or PIPs produced naturally by non-GE organisms are exempt. EPA regulates the pesticides but not the plant itself. Use permits are issued for field testing. Applicants must register pesticidal products prior to their sale and distribution and EPA may establish conditions for use as part of the registration. The EPA sets tolerance limits for residues of pesticides on and in food and animal feed under the Federal Food, Drug and Cosmetic Act (FFDCA). The EPA, through its Office of Prevention and Toxic Substances Biotechnology Programme, regulates products of biotechnology through its interpretation that organisms are 'chemical substances' under the Toxic Substances Control Act (TSCA). Developers must notify the EPA 90 days prior to manufacture or 60 days prior to field testing of a product regulated by TSCA. According to the EPA, the PRSV *CP* transgene is a pesticide because it confers resistance to plant viruses. Thus, it was subjected to tolerance-levels evaluation in the plant. In the permit application, we petitioned for an exemption from tolerance levels of the CP produced by the transgenic plant. We contended that the pesticide (the *CP* gene) was already present in many fruits consumed by the public, since much of the papaya eaten in the tropics is from PRSV-infected plants. In fact, we had earlier used cross-protection to control PRSV and fruits from these trees were sold to consumers. Furthermore, there is no evidence to date that the CP of PRSV or other plant viruses is allergenic or detrimental to human health in any way. Finally, measured amounts of CP RNA or protein in transgenic plants were much lower than those of infected plants. An exemption from tolerance to lines 55-1 was granted in August 1997.

FDA, through its Centre for Food Safety and Nutrition (CFSN), is responsible for ensuring the safety and proper labelling of all plant-derived foods and feeds, including those developed through bioengineering under the FFDCA. However, the true legal responsibility for food safety falls upon the developer. The FDA is also concerned with possible 'food additives',

substances introduced into food that are not pesticides or not generally recognized as safe (GRAS). This agency follows a consultative process whereby the investigators submit an application with data and statements corroborating that the product is not harmful to human health. Evaluation by the FDA is based on the idea of 'substantial equivalence', meaning the nutritional or toxin content of a GE food is within the range normally found in conventional varieties. If the GE food is substantially equivalent, then it is regulated in the same way as non-GE food. GE foods which are nutritionally different, such as canola or soybeans with altered oil content, require labelling. In the evaluation of transgenic papaya 55-1 by the FDA, several aspects were considered: the range of concentration of some important vitamins, including vitamin C; the presence of *uidA* and *nptII* genes; and whether transgenic papaya had abnormally high concentrations of benzyl isothiocyanate (BITC). This latter compound has been reported in papaya (Tang, 1971). FDA approval was granted in September 1997.

Intellectual property rights

In the USA, a transgenic product cannot legally be commercialized unless it is fully deregulated and until licenses are obtained for the use of the intellectual property rights for processes or components that are part of the product or that have been used to develop the product (Gonsalves, 1998). The processes in question were the gene gun and PDR, in particular, CP-mediated protection. The components were translational enhancement leader sequences and genes (*nptII, uidA* and *CP*). This crucial hurdle involved legal and financial considerations beyond our means and expertise. These tasks were taken up by the industry's Papaya Administrative committee (PAC) and its legal counsel, Michael Goldman. The license agreements were obtained from all parties in April 1998, allowing the commercial cultivation of the papaya or its derivatives in Hawaii only. On 1 May, 1998 seeds were distributed free to growers who qualified by watching an educational video and signing an agreement that restricted growing of transgenic papaya only in Hawaii. Fruits can be sold outside Hawaii, provided that the importing state or country allows the importation and sale of transgenic papaya (Gonsalves, 1998). Thus, commercialization of SunUp and Rainbow began in Hawaii in 1998, 8 years after transgenic papaya 55-1 was first created. This followed deregulation by APHIS, which occurred in 1996, 4 years after the first field trials and deregulation by the EPA and FDA in 1997.

Impact of Rainbow on Papaya Production

The impact of the transgenic papaya on the papaya industry can be seen by its rapid rate of adoption in Puna, expressed as the percentage of bearing (actively producing) area of Rainbow and Kapoho (Table 19.3). In 2000,

Table 19.3. Production of transgenic papaya in Puna, Hawaii. (Modified from Tripathi *et al.*, 2006.)

Year	Bearing hectare	% Kapoho	% Rainbow	Production
1998	663.7	100	0	12,134
2000	485.6	38	56	15,399
2001	677.8	45	47	18,275
2002	603.0	53	40	16,275
2003	574.7	45	47	16,209
2004	493.7	35	56	13,606
2005	511.9	22	66	12,206

Bearing area in Puna of non-transgenic Kapoho and transgenic Rainbow in hectares (ha) and the relationship to production (× 1000 kg) of fresh fruit utilized from the year 2000. Data for 1998 were included for reference. Data were compiled from USDA Statistical Reports of Papaya grown in Hawaii (www.nass.usda.gov/hi).

production in Puna rebounded to 15,399 t from the production low of 11,617 t in 1999 (Table 19.4). This coincided with the first recorded harvests from Rainbow, which comprised 56% of the bearing area that year, while Kapoho comprised 38%. In 2001, Puna papaya production peaked at 18,275 t with a near equal percentage of bearing area of Rainbow and Kapoho. In recent years, the percentage of bearing area of Rainbow has seen an upward trend to the current high of 66% as of 2005. These data indicate that Rainbow has been fully embraced by commercial growers as a popular and profitable cultivar. While the production levels for 2004 and 2005 seem to suggest a downward decline, the actual yields on a per hectare basis have been consistently higher after the introduction of Rainbow compared to yields before its introduction in 1998.

The impact of PRSV on papaya production can be observed by examining the contribution of Puna to Hawaii's total fresh papaya production. In 1992, Puna produced 24,045 t or 95% of the state's 25,310 t of fresh papaya (Table 19.4). Puna's production remained high for 2 years following the discovery of PRSV as a result of massive efforts to control the spread of the virus. In the years of total production decline caused by PRSV from 1995 to 1999, there was a concomitant sharp decrease in the percentage of papaya harvested from Puna. However, since the first recorded harvest of Rainbow in 2000, Puna's contribution to production has steadily and continually climbed, reaching 88% of the state's total in 2005. A substantial portion of this increase as noted above is due to the contribution of Rainbow to the bearing area. The data seem to indicate that acquisition of virus-resistant Rainbow has had a stabilizing effect on papaya production in Puna and subsequently on the industry as a whole. In 2005, when production in Puna reached a recent low of 12,206 t, it still accounted for 88% of Hawaii's total fresh

Table 19.4. Fresh papaya production in the state of Hawaii and in the Puna district from 1992–2005[a]. (Modified from Tripathi *et al.*, 2006.)

	Fresh papaya utilization in Hawaii		
Year	Total (× 1000 kg)	Puna (× 1000 kg)	%
(Virus in Puna) 1992	25,310	24,045	95
1993	26,399	25,079	95
1994	25,492	25,186	99
1995	19,006	17,788	94
1996	17,146	15,511	90
1997	16,193	12,614	78
(Transgenic seeds released) 1998	16,148	12,134	75
1999	17,872	11,617	65
2000	22,793	15,399	68
2001	23,587	18,275	77
2002	19,368	16,275	84
2003	18,507	16,209	87
2004	15,467	13,606	88
2005	13,925	12,206	88

[a]Data were compiled from USDA Statistical Reports of Papaya grown in Hawaii (www.nass.usda.gov/hi).

papaya production, due to low overall productivity in the entire state. This reinforces the important role of Rainbow, since even aside from the negative impact of PRSV on papaya production, other variables such as the weather, rising costs of maintaining healthy orchards and low prices can also profoundly and negatively affect productivity and the health of the papaya industry.

Environmental Risk Issues

Rainbow and SunUp papaya are among the few transgenic crops accepted for commercial production that carry the virus-resistance trait. Due to their potential impact on the environment and human health, the development and release of virus-resistant transgenic plants expressing viral genes continues to raise special concerns beyond general contentions against transgene technology. The major concerns are heteroencapsidation, recombination, transgene flow to wild relatives and potential weediness of virus-resistant plants (Fuchs *et al.*, 1998, 1999; Thomas *et al.*, 1998; Lin *et al.*, 2003; Vigne *et al.*, 2004; Fuchs and Gonsalves, 2007; Chapter 18, this volume). In this section, we will only discuss these concerns as they relate to the transgenic papaya in Hawaii.

Heteroencapsidation

Heteroencapsidation refers to the encapsidation of the genome of a challenge virus by the CP protein subunits expressed in a transgenic plant. Heteroencapsidation has been documented in transgenic herbaceous plants (Osburne *et al.*, 1990; Holt and Beachy, 1991; Candelier-Harvey and Hull, 1993; Lecoq *et al.*, 1993; Hammond and Dienelt, 1997; Fuchs *et al.*, 1998; Fuchs and Gonsalves, 2007).

Regarding the transgenic papaya in Hawaii, heteroencapsidation is of little or no consequence because papayas in Hawaii are infected only by PRSV. There have been reports of the tospovirus *Tomato spotted wilt virus* in Hawaii, but it is not common. The only other major aphid-transmitted potyvirus to infect papaya is *Papaya leaf distortion mosaic virus* (PLDMV), but it does not occur in Hawaii. The PLDMV CP protein is not serologically related to that of PRSV (although they both belong to the same group) which may limit heteroencapsidation in nature. Evidence from our laboratory suggests that the mechanism of resistance in Rainbow and SunUp is via PTGS, with very low expression of both the transgene CP RNA and protein, much lower in fact than that observed upon PRSV infection in a non-transgenic plant. Thus, the likelihood of heteroencapsidation and increased risk beyond that which occurs during mixed infections in nature would presumably be low.

Recombination

Recombination of a viral transgene with an incoming virus can potentially lead to a genetic change which might allow the proliferation of novel recombinants (AIBS, 1995). Recombination is a potential environmental risk issue since it can theoretically result in changes in pathogenicity, such as increased virulence or impact on non-target organisms due to possible changes in host specificity (Fuchs and Gonsalves, 2007). In relation to the transgenic papaya in Hawaii, we have no evidence of recombination occurring under field conditions. A major roadblock to recombination occurring in the transgenic papaya grown in Hawaii is that, so far, none of the PRSV isolates from Hawaii tested have been able to overcome transgenic papaya resistance.

Notwithstanding, the development of a system to produce infectious viral transcripts of PRSV *in vitro* (Chiang and Yeh, 1997) has provided a unique opportunity to begin to functionally identify viral gene segments involved in various functions including pathogenicity. In practice, this was accomplished by construction of *in vitro* transcription templates that consisted of genetically engineered, chimeric PRSV genomes with the normal complement of genes, but composed of segments from two or more parental strains. The recombinant PRSV approach was used for the identification of gene segments involved in pathological differences between YK, a PRSV isolate from Taiwan and the Hawaiian isolate HA on transgenic Rainbow

and SunUp papaya. It had been assumed that the YK strain overcame resistance of Rainbow and SunUp mainly due to the low homology (89.9%) of its *CP* gene to the HA 5-1 *CP* transgene. To test this assumption, chimeric constructs were made with the HA virus containing all or portions of its *CP* gene and 3' end non-coding region replaced with that of the corresponding regions of YK (Chiang *et al.*, 2001). Although recombinants with the entire *CP* gene of YK did indeed cause severe symptoms on Rainbow, these were not as severe as the wild type YK genome. Studies utilizing recombinant virus composed of segments from severe and mild strains of PRSV also indicated that the HC-Pro gene plays an important role in viral pathogenicity and acts as suppressor of the gene silencing defence mechanism in papaya (Tripathi *et al.*, 2003, 2004; Yeh *et al.*, 2003; Bau *et al.*, 2004).

These functional experiments show that the CP is not the sole determinant for pathogenicity, but that pathogenic properties of PRSV are governed by the collective contribution of multiple viral genes. In cases such as viral transmission, the *CP* gene functions in this process may require specific interactions with other gene(s) which must already be present in the challenge virus. One interpretation of this data is that should recombination occur, the CP originating from the transgene would only be expected to function in pathogenic processes in the context of closely related or near identical viral genomes which would consequently have similar properties to PRSV.

Transgene flow to wild relatives

One of the major environmental safety issues over virus-resistant transgenic crops is gene flow. Gene flow is not a risk specific to the virus-resistance trait, but its impact on recipient plants could be affected by additional factors, such as plant virus and virus vector prevalence in the environment. Wild relatives of cultivated crops can acquire host genes and/or transgenes through pollen flow and their progeny resulting from gene transfer can exhibit undesired characteristics if the transferred genes provide them with a selective advantage (Fuchs and Gonsalves, 2007).

In Hawaii, there are neither wild relatives nor non-domesticated *Carica papaya*. Even if wild relatives (previously classified in the genus *Carica* but now classified in the genus *Vasconcellea*) were to exist in Hawaii, they are not sexually compatible with *Carica papaya* (Gonsalves *et al.*, 2006). Thus, in Hawaii, there is no risk that gene flow will occur between transgenic papaya and non-domesticated papaya or wild relatives.

Weediness of virus-resistant papaya in Hawaii

PRSV was discovered in Hawaii in the 1940s. In the Territorial records prior to the 1940s, papaya was not listed as a weed. This indicates that

even in the absence of disease caused by PRSV, papaya is not nor does it become weedy. Similarly, addition of the virus-resistant trait should not cause papaya to become weedy. Indeed, observations since release of the virus-resistant transgenic papaya in Hawaii confirm that it is not a weed.

Management Issues

In this section, we discuss management issues relating to virus-resistant transgenic crops, citing examples directly from the practices employed in the commercial production of Rainbow and SunUp papaya in Hawaii. The important management issues discussed include understanding, guarding and extending the durability of PRSV resistance of transgenic papaya, factors and measures allowing the production of non-transgenic papaya and maximizing the utility of transgene resistance through cultivar development. The issue of gene flow is discussed here in the context of 'coexistence' in the production of Hawaii's non-transgenic and transgenic papaya as a management issue.

Durable resistance

The breakdown of resistance is of concern for any virus-resistant plant, whether derived from conventional breeding or through transgenics. It is a concern for managing or prolonging the effective period of the transgenic papaya to the point that its economic benefits are maximally realized, particularly in light of the energy expended for its deregulation. As mentioned above, greenhouse inoculations of transgenic 'Rainbow' showed that the transgenic papaya was resistant to only some of the strains of PRSV collected from outside the USA (Tennant *et al.*, 2001). Thus, it is critical to constantly monitor the introduction or emergence of viral strains that could overcome the resistance of the transgenic plants, and accordingly develop a proactive strategy. This practice is extremely important because it takes a long time to develop resistant plants. Since the development of Rainbow, personnel in Hawaii have been continually testing for the breakdown of resistance by routinely challenging Rainbow with locally collected PRSV isolates as well as monitoring Rainbow fields for susceptible plants. In the 8 years since its commercial release, no breakdown in resistance to local isolates has been observed (Ferreira and Gonsalves, 2006).

Performance studies on SunUp and other transgenic papaya have shown that resistance can be broadened to other geographical isolates by increasing the transgene dosage (Tennant *et al.*, 2001). Thus, in recent years, introgression and doubling of the 55-1 transgene into popular local cultivars has been employed as a approach to help sustain disease resistance durability as well as variety in the market (Gonsalves *et al.*, 2006) (see also the section 'Development of new cultivars'). Another important

part of our disease management strategy has been to remain proactive and ready for the incidence of resistance breakdown by developing new transgenic lines that are resistant to PRSV strains from outside of Hawaii in addition to local strains (Fermín and Gonsalves, 2003; Gonsalves and Ferreira, 2006).

Production of non-transgenic papaya

One of the major contributions that the transgenic papaya has made to the Hawaiian papaya industry is that it has revived lucrative production of non-transgenic papaya (Gonsalves and Ferreira, 2003). This has occurred in several ways. First, the initial large-scale planting of transgenic papaya in established farms along with the elimination of abandoned virus-infected fields drastically reduced virus inocula and thus allowed for strategic planting of non-transgenic papaya in areas that did not have infection. As early as 1999, the HDOA instituted a plan to ensure the production of non-transgenic papaya in an area known as Kahuawai which is physically isolated from established fields in Puna. Kahuawai was also protected to some extent from aphid vectors carried over from infected fields since the prevailing winds came from the direction of the ocean which bordered the field (Gonsalves and Ferreira, 2003). Growers who followed the recommended practices of monitoring for and rogueing of infected plants were able to economically produce 'Kapoho' without major losses from PRSV. Second, although definitive experiments have not been carried out, it seems that transgenic papaya can provide a buffer zone to protect non-transgenic papayas that are planted within the confines of the buffer. Our reasoning is that viruliferous aphids feeding on transgenic papaya will be purged of virus before travelling to the non-transgenic plantings within the buffer. Thus, growing transgenic and non-transgenic papaya in relatively close proximity may function in management of PRSV infection of non-transgenic papaya. For reasons stated above, production of non-transgenic papaya in Hawaii continues today, and in fact is lucrative and vital, since Japan, which represents a significant share of the Hawaiian papaya export market, has a zero tolerance for transgenic papaya.

An ever-present and continual challenge in maintaining non-transgenic papaya production in Puna is to prevent the significant build-up of virus. This is because PRSV is still around and therefore strict attention is required in planting non-transgenic papaya fields in locations isolated from other non-transgenic fields, and in the timely elimination (rogueing) of infected trees and non-transgenic plantings that are no longer in production to prevent the build-up of virus inoculum. Although important, these simple factors are often not practiced when there are no obvious signs for resurgence of PRSV. It is hoped that people will not forget the tremendous damage that PRSV caused to Hawaii's papaya industry during the period 1992–1998.

Coexistence

Coexistence, the growing of transgenic and non-transgenic papaya in practical proximity to each other such that they can be raised with minimal transfer of genetic characteristics from transgenic to non-transgenic, is in fact, being practiced in the Hawaiian papaya industry today. This situation has been brought about since both transgenic and non-transgenic papayas are necessary for the Hawaiian papaya industry, especially in the growing of organic papaya and in maintaining the Japanese market which at present does not accept transgenic papaya since the latter has not been deregulated in Japan. It should be noted that the USA does not require deregulated crops such as Rainbow and SunUp to be grown in specified locations within the USA.

One means by which practical tools have been introduced to the Hawaiian papaya industry for monitoring and managing non-transgenic papaya production is through adoption of an Identity Preservation Protocol (IPP) (Camp, 2003). This voluntary programme was established by the HDOA at the request of Japanese papaya importers. Documented compliance to the regulations laid out in the IPP allows farmers and shippers to receive a certification letter from the HDOA that accompanies each papaya shipment. The incentive for participation in the programme is that the IPP certification letter allows papaya shipments to be distributed while Japanese officials perform tests for possible contaminating transgenic papaya from samples of the shipment. Shipments without the certification letter must be held until the tests are completed, which may take anywhere from a few days to a week, during which time the fruit may lose quality and marketability.

In order to obtain an IPP certification letter, the non-transgenic papaya must come from papaya orchards approved by the HDOA. Every tree in the orchards in question must be derived from seeds produced in approved, non-GMO fields and each tree must be tested by the applicant and found to be negative for the transgene-linked GUS activity. A papaya-free zone of at least 4.5 m must also separate the non-transgenic orchard. The applicant must subsequently submit detailed records of the transgene detection tests to the HDOA. Prior to final approval of the field by the HDOA, the applicant must in addition randomly test one fruit from 1% of the papaya trees in the field for presence of the transgene. Detailed postharvest protocols for minimizing the chance of contamination of non-transgenic papaya with transgenic papaya, including procedures such as the random testing of papaya prior to packing, must also be submitted to the HDOA and adhered to. If all criteria are met, the certification letter from HDOA will accompany the shipment stating compliance with a properly conducted IPP.

In summary, coexistence is being routinely practiced in Hawaii's papaya industry. The scheme of IPP has proved workable and economical as papaya is still being shipped routinely to Japan without evidence of transgenic fruits.

Development of new cultivars

The successful control of PRSV by Rainbow papaya has spawned the development of new virus-resistant varieties, which have opened new market niches and has enabled the expansion of profitable production of papaya in other regions of Hawaii (Gonsalves *et al.*, 2004). In addition to SunUp and Rainbow, two new cultivars, Poamoho Gold and Laie Gold, were developed primarily for growers on the island of Oahu (Fitch *et al.*, 2002). In contrast, prior to 1998, Hawaii had only one dominant cultivar, the non-transgenic Kapoho and a small areage of Sunrise. Today, Hawaii has SunUp, Rainbow, Kapoho, Sunrise, Laie Gold and Poamoho Gold.

Efforts to Deregulate Transgenic Papaya in Canada and Japan

Although a major constraint to papaya production in Hawaii has been eliminated with the introduction of PRSV-resistant transgenic plants, Hawaii's papaya industry still faces a number of challenges. Some of these challenges have been mentioned previously and include maintaining production of non-transgenic papaya, the durability of the resistance of transgenic papaya, concerns particularly of organic growers that their crops will be contaminated by pollen flow and the general controversy over GMOs. In this section, we address the steps that have been and are being taken to gain market share of transgenic papaya in Canada and Japan.

Canada

Canada accounts for 11% of Hawaii's papaya export market. Canada considers foods derived from GMOs as 'novel foods' and importation requires review and approval by Health Canada (HC), the government organization responsible for food safety. The Canadian Food Inspection Agency (CFIA) and Environment Canada (EC) are two other agencies involved in other aspects of regulation and approval of GMO-derived products. Health Canada approved the import of 'SunUp' and 'Rainbow' transgenic papaya for food purposes only in January 2003. Labelling of the approved transgenic papaya imported into Canada is not required. The data used for the nutritional assessment of the transformant line 55-1 included fruit composition (total soluble solids, carotenoids, vitamin C and minerals), which were within the range found in fruit of non-transgenic cultivars grown in Hawaii. In the toxicology assessment, PRSV CP was not considered a 'novel' protein due to the history of human consumption of PRSV-infected fruit without adverse health effects (http://www.hc-sc.gc.ca/fn-an/gmf-agm/appro/papaya_e.html).

Japan

Currently, Japan accounts for 20% of Hawaii's papaya export market. As mentioned above, the application process for sale of transgenic papaya, specifically derivatives of 55-1, in Japan has not yet been approved, so at present Hawaii exports only non-transgenic papaya to Japan. Obviously, approval for the sale and shipment of transgenic papaya will circumvent much of the concern and consequences of accidental introduction of transgenic papaya into Japan. Recently, government agencies such as the US Foreign Agricultural Service (FAS) have also expressed enthusiasm in support of this goal because of their interests in promoting US biotechnology in other countries. To this end, efforts to allow transgenic papaya into Japan were initiated by the then Papaya Administrative Committee or PAC (later replaced by the present day Hawaii Papaya Industry Association or HPIA) soon after the transgenic papaya was commercialized in Hawaii, with the researchers taking the lead in developing the petition. For the application to allow import of transgenic papaya to Japan, both food for human consumption and environmental safety issues are being evaluated. The petition to the Japanese Ministry of Agriculture, Forestry and Fisheries (MAFF) was approved in 2000, while a petition initially submitted to the Japanese Ministry of Health, Labour and Welfare (MHLW) in 2003 has undergone revision and is in the process of evaluation. Since the initial petitions were filed, additional information has been requested from both Japanese ministries due to subsequent adoption of new policies on the regulation of GMOs.

Currently, advisory committees and expert panelists of the Ministry of Agriculture, Forestry and Fisheries (MAFF) and the Ministry of the Environment (MOE) perform environmental risk assessment and safety evaluations. The environmental safety policies follow the Biosafety Protocol implemented in 2004 by Japan's 'Law Concerning the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Living Modified Organisms' or 'Cartagena Law' (Sato, 2006). Japan's laws and policies on environmental risk assessment follow along the lines of a UN agreement called The Cartagena Protocol on Biosafety (CPB) to which it is a member state (http://www.biodiv.org/biosafety/default.aspx). The CPB itself was adopted in 2000 and put into force in 2003. It is a supplementary agreement of the UN Environmental Programme's (UNEP) Convention on Biological Diversity (CBD; http://www.biodiv.org/default.shtml). CBD is an international treaty for the development of national strategies for the conservation and sustainable use of biological diversity (sustainable development) adopted at the UN Conference on Environment and Development (Earth Summit) in Rio de Janeiro in 1992 (http://www.un.org/geninfo/bp/enviro.html). The CPB is based on the 'precautionary principle' and covers regulations dealing with the management or control of risks associated with transfer (particularly across borders), handling, and use of GMOs, termed Living Modified Organisms (LMOs), activities that might adversely affect the environment.

Some of the information and data requested by the MAFF and MOE included alleopathy on plants and soil microbial communities, physiology of papaya, aetiology of PRSV, and pollen biology relating to the potential for transgene contamination by pollination. One of the additional areas we were asked to address was the potential for recombination of transgene encoded sequences with other viruses and its possible impact.

An expert panel and committee of the Food Safety Commission (FSC) are evaluating issues relating to safety of food for human consumption. Their conclusions will be reported to the Ministry of Health, Labour and Welfare (MHLW) under Japan's Food and Sanitation Law. Japan's current policies regarding food safety of GMOs were established in 2004 and draw upon some of the guidelines presented by the Ad Hoc Intergovernmental Task Force on Food Derived from Biotechnology of the Codex Alimentarius 'food code' Commission (http://www.who.int/foodsafety/biotech/codex_taskforce/en/index.html) (http://www.fsc.go.jp/senmon/idensi/gm_kijun_english.pdf). The commission is a subsidiary body of the UN Food and Agriculture Organization (FAO) and the World Health Organization (WHO).

The FSC requested information to determine substantial equivalence between non-transgenic and transgenic papaya for substances including BITC and papain, papaya fruit protein profiles, in addition to allergenicity related studies including PRSV CP heat stability and stability in simulated intestinal and gastric fluid.

Detailed molecular genetic data including Southern hybridization analysis of 55-1 using probes covering the entire transformation plasmid, PCR of the insert border regions and sequence of the insertions and flanking genomic DNA were required and prepared for submission to both the MHLW/FSC and the MAFF/MOE. In addition, open reading frame (ORF) analysis followed by Blast searches to all inclusive and allergen-specific databases were performed on the inserts and flanking genomic DNA to determine the potential expression of toxic or allergenic proteins. These bioinformatic data were submitted to the MHLW/FSC and the MAFF/MOE. Such detailed molecular analysis of the transgene insertion event was not required by the relevant US regulatory agencies.

During review for potential allergenicity of transgene-derived proteins, questions were raised on the potential allergenicity of the PRSV CP. According to the FAO/WHO, 2001 discussion (FAO/WHO, 2001), matches of six amino acids of a protein to known allergens make it a candidate for being an allergen. Using this criterion, Kleter and Peijnenburg (2002) determined that there was a single 6 amino acid match of the PRSV CP to a proposed allergen ABA 1, a protein of the human parasite *Ascaris lumbricoides* or the pig parasite *Ascaris suum*. In response, we claimed that for several reasons, the amino acid homology between PRSV CP and ABA 1 is not relevant with regards to allergenicity; the amino acid sequence is not repeated in the CP sequence like allergenic epitopes usually are; therefore, it would not be expected to trigger the IgE response associated with allergens. The ABA 1 proposed allergenic peptide was found to not

be inherently allergenic outside the context of other *Ascaris* proteins (Paterson *et al.*, 2002) and indeed, is not among the officially recognized allergens found at the International Union of Immunological Societies (IUIS) web site (http://www.allergen.org).

If the various agencies approve the Food Safety and Environmental Safety submissions, the form of labelling of the transgenic papaya will then be decided among subcommittee(s) of the MAFF and MHLW followed by notification of the entire approval package to the World Trade Organization (WTO).

Receiving approval for the importation of transgenic papaya into Japan would have huge benefits to Hawaii's papaya industry and would also advance the case for the acceptance and development of transgenic products outside the USA. Following approval, shippers of non-transgenic papaya would likely still have to label their cargo as such, but are also likely to be allowed to continue shipments in cases where errors occur within certain defined tolerance limits, a situation that contrasts with the present day strict zero tolerance policy against transgenic papaya.

The introduction of transgenic papaya fruit to Japan will allow consumers to make a personal choice, serving as a real life example for consumer acceptance of fresh GMO products outside of the USA. Since the transgenic Hawaiian papaya was not developed with support from multinational corporations and is not a major commodity transgenic crop, consumer acceptance should not be clouded by media hype and sentiment against the dominance of multinational corporations or international trade issues. Rather, it is hoped and anticipated that product acceptance will be influenced by factors such as quality, price, advertising, and philosophy of the individual consumer. In this respect, the transgenic papaya will be a ground-breaking biotechnology for the greater, worldwide consumer and governmental acceptance of fresh transgenic products.

Conclusions

In this chapter, we have presented practical accounts on the steps taken to bring about the commercialization of virus-resistant transgenic papaya based on the PDR approach in Hawaii. More than 8 years after its introduction in 1998, the transgenic virus-resistant papaya continues to play a vital role in the Hawaiian papaya industry in the practical and effective management of PRSV, which is essential for the economic production of papaya. Similarly, the more widespread implementation of virus-resistant transgene technology in papaya and other crops, including regional and underrepresented crops, should have a great impact on the management of virus diseases as well as on the economies and health of the local communities not currently enjoying its benefits. Understanding the actual risks and safety issues regarding the implementation of transgene technology under real life conditions and acceptance of the concept are important factors in the development of sensible regulation and the greater adoption of the technology.

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